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On the maternal transfer of 4-aminobiphenyl in rats

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The potential for 4-aminobiphenyl (4-ABP) to be transferred from circulating blood into the milk of lactating Sprague–Dawley rats was determined. The distribution of ^{14}C-labeled 4-ABP into milk was examined at time intervals of <1, 20, 60, 120, 240 and 480 min after i.v. dose administration. Elimination of radioactivity from blood and milk was determined to be biphasic. The levels of 4-ABP and/or metabolites were lower in milk than in blood at all time points examined. The levels of radioactivity detected in blood declined less rapidly than in milk. That is, the percent of the dose per ml of blood declined from 0.81–0.45, while the percent of the dose per ml of milk declined from 0.38–0.06 during the 8 h time period. The radioactivity present in milk was partially extractable with ethyl acetate with 43% of the radioactivity being extractable at the earliest time point while only 16% was extractable after 8 h. The level of radioactivity associated with the protein precipitate of the milk samples increased from 4–21% within 4 h after treatment. The potential of 4-ABP or its metabolites to exert a genotoxic effect on newborn pups *via* maternal transfer was also examined. Dams were treated on day 1 post partum and then daily with 4-ABP (10 mg/kg) in corn oil or corn oil alone for 2 weeks. Each experimental group had four litters of pups each containing 5 pups. Pups were sacrificed at 15 days of age, separated by sex and the levels of 4-ABP:DNA adducts in liver determined using ^{32}P-postlabeling. DNA adduct profiles were similar between male and female pups with total adduct levels of 332 and 338 fmol of adducts/mg of DNA, respectively. These results indicate that the genotoxic effects of 4-ABP can be transmitted from exposed dams to the nursing offspring.

Introduction

The occurrence and carcinogenic activity of 4-aminobiphenyl (4-ABP*) has previously been reviewed (1,2). Recently, methods have been developed to determine the dosimetry of 4-ABP in humans by analysis of its binding to hemoglobin (Hb) (3–5). In studies performed with humans, 4-ABP was detected covalently bound as the sulfinic acid amide to Hb at levels ranging from 10–260 pg per g (5). The mean level of 4-ABP binding to hemoglobin in smokers was 154 pg per g Hb as compared to 28 pg per g Hb for nonsmokers. These data are not unexpected in view of the fact that 4-ABP is present in mainstream cigarette smoke at levels ranging from 2–5 ng/cigarette (6). Several aromatic amines, including 4-ABP, are suspect human bladder carcinogens. It has been suggested that the presence of these aromatic amines in tobacco smoke may be a factor responsible for the elevated risk of cigarette smokers to develop bladder cancer.

Twenty to thirty percent of nursing mothers are cigarette smokers (7,8). In recent studies we have shown that the tobacco-specific carcinogens *N'*-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) as well as benzo[*a*]pyrene (BaP) are transferred into the milk of lactating rats. The concentrations of these tobacco-related carcinogens in milk were similar to those detected in the circulating blood of the treated dam (9). In this study we evaluated the extent to which 4-ABP and/or its metabolites are transferred from circulating blood into the milk of lactating Sprague–Dawley rats. ^{32}P-Post-labeling analysis of DNA adducts formed from nonradioactive carcinogens permits the detection of adducts from microgram quantities of DNA (10,11). This approach has been used to examine DNA adduct formation in various tissues following 4-ABP exposure (12,13). This methodology has also recently been employed to detect transplacental DNA damage induced by safrole, benzo[*a*]pyrene, and 4-ABP (14). Using this technique, we have also examined the potential for DNA adduct formation in the livers of exposed male and female pups as a result of maternal transfer of 4-ABP and/or its metabolites.

Materials and methods

Chemicals

[Ring-U-^{14}C]-labeled 4-ABP (60 mCi/mmol) was purchased from Chemsyn Science Laboratories (Lenexa, KS). Emulphor EL 620 was obtained from GAF Corp. (Linden, NJ). Nembutal (50 mg/ml saline) was purchased from Abbott Laboratories (Chicago, IL). Oxytocin (20 U/ml) was obtained from Butler Company (Columbus, OH). Triton X-100 and *t*-butylhydroperoxide (as a 70% aqueous solution) were purchased from Sigma Chemical Company (St Louis, MO). Monofluor was obtained from National Diagnostics (Manville, NJ). Chem Elut extraction tubes (#1005) were obtained from Analytichem International (Harbor City, CA).

Bioassay to determine extent of transfer into milk

Female Sprague–Dawley rats with their natural first born litters were purchased from Charles River Laboratories, Kingston, NY. The litters were received when the pups were 4–5 days old. Each dam together with her litter was housed in a solid-bottom polycarbonate cage and fed Purina lab chow *ad libitum*. Animals were kept under standard conditions (22 ± 2°C; 50 ± 10% relative humidity; light–dark cycle, 12–12 h).

Dams were separated from the pups for approximately three hours prior to treatment on the eleventh day post partum. The levels of 4-ABP and its metabolites were determined in the blood and milk of four dams at each time interval of <1, 20, 60, 120, 240 and 480 min after i.v. administration (tail vein) of 83 nmol of ^{14}C-labeled 4-ABP (60 mCi/mmol) in 0.5 ml of 30% Emulphor 620 in water. The dams used at each time interval were anesthetized using Nembutal (35 mg/kg) ~15 min prior to removal of blood and milk samples. From each animal 200 μl of blood was obtained from the retrobulbar venous plexus before and after milking. Milking was performed as previously described (9). The time required to milk each dam ranged from 3–13 min, with the average time being 7.3 min. The volumes of milk obtained from each of the dams are listed in Table I. In a limited study the effect of dose of 4-ABP on the extent to which it could be transferred into the milk and blood of lactating rats was investigated. In this study four dams were injected with 27 μmol of ^{14}C-labeled 4-ABP (0.3 mCi/mmol). Blood and milk samples were collected as described above 60 min after dose administration.

*Abbreviations: 4-ABP, 4-aminobiphenyl; Hb, hemoglobin; NNN, *N*-nitrosonornicotine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; BaP, benzo[*a*]pyrene.

Table I. Time course of the distribution of ^{14}C-labeled 4-aminobiphenyl in the milk and blood of lactating rats[a]

Time (min)	Sample no.	Wt of dam (g)	Volume of milk (ml)	4-ABP in milk per ml (% of dose)	4-ABP in blood per ml (% of dose)
<1.0	1	415	4.65	0.32	0.71
	2	352	3.05	0.38	0.93
	3	397	3.00	0.39	0.73
	4	364	3.00	0.42	0.86
Average ± SE		382 ± 13	3.43 ± 0.41	0.38 ± 0.02	0.81 ± 0.05
20	5	381	3.80	0.16	0.77
	6	424	3.40	0.15	0.72
	7	374	1.00	0.12	0.69
Average ± SE		393 ± 16	2.73 ± 0.87	0.14 ± 0.01	0.73 ± 0.02
60[b]	8	436	3.85	0.07	0.58
	9	377	3.90	0.08	0.59
	10	321	3.05	0.10	0.53
	11	408	3.65	0.07	0.48
Average ± SE		386 ± 25	3.61 ± 0.20	0.08 ± 0.01	0.55 ± 0.03
120	12	387	3.00	0.09	0.49
	13	321	3.20	0.07	0.61
	14	376	2.90	0.10	0.30
	15	332	4.10	0.07	0.39
Average ± SE		355 ± 16	3.30 ± 0.27	0.08 ± 0.01	0.52 ± 0.05
240	16	367	4.85	0.08	0.52
	17	399	4.50	0.09	0.46
	18	335	3.40	0.13	0.37
Average ± SE		367 ± 18	4.25 ± 0.43	0.10 ± 0.01	0.45 ± 0.04
480	19	382	5.20	0.06	0.50
	20	429	3.80	0.07	0.32
	21	426	2.90	0.05	0.39
	22	362	2.20	0.05	0.58
Average ± SE		400 ± 17	3.52 ± 0.64	0.06 ± 0.01	0.45 ± 0.06

[a]The data summarized in this table reflect the results obtained from the treatment of each dam with 5.0 μCi of 4-ABP (60 mCi/mmol).
[b]Four dams were also treated with 5.2 μCi of ^{14}C-labeled 4-ABP (0.3 mCi/mmol). The average percentage of the dose detected in blood and milk 60 min after dose administration was 0.81 ± 0.05 and 0.22 ± 0.02 percent per ml, respectively.

Analysis of 4-ABP in blood and milk

Blood samples (20 μl) obtained from the dams administered ^{14}C-labeled 4-ABP or the vehicle alone were digested and analyzed using an improved method (D.Evans, personal communication) compared to that previously employed (9). Blood samples (200 μl) were initially treated with 0.5 ml of 2.0 N NaOH and placed in a shaking incubator at 55°C for 2 h or until all solid residues were dissolved. To each sample was added 0.5 ml of a 70% aqueous solution of *t*-butylhydroperoxide. These solutions were allowed to stand overnight prior to the addition of 0.2 ml of a 50% acetic acid and 0.5 ml of scintillation grade Triton X-100. After addition of Monofluor (10 ml), samples were left to stand in the dark overnight prior to being counted. Blood values represent the average from blood samples taken before and after milking. Radioactivity in milk samples was determined by direct scintillation counting by combining 1.0 ml aliquots of milk with 20 ml of monofluor scintillation fluid. Samples were counted within 30 min after mixing to assure homogeneity as a precipitate develops after several hours at room temperature. Data were further analyzed with PKCALC, a computer program used to estimate the biological half-life of total radioactivity in the blood and milk (15).

Analysis of protein bound and ethyl acetate extractable residues of ^{14}C-labeled 4-ABP in milk

Radioactivity in milk was further characterized as being protein bound or ethyl acetate extractable using the following procedure. Approximately 2 ml of milk samples was applied to a 5.0 ml Chem Elut extraction tube which had been previously filled with 25 ml of acetate. The initial aqueous acetate solution and an additional 50 ml of ethyl acetate were eluted through the column by employing a slight vacuum. The protein which precipitated from the milk sample during the extraction remained on the cotton gauze at the top of the Chem Elut tube. The ethyl acetate eluent was concentrated *in vacuo* to a final volume of 10 ml. The amount of radioactivity in the ethyl acetate eluent was determined by liquid scintillation counting. The protein which was retained on the cotton gauze was removed and placed in a scintillation vial. This material was treated with 3.0 ml of 1.0 N NaOH for 72 h at 55°C with shaking. After addition of 0.2 ml of 70% aqueous *t*-butylhydroperoxide, these samples were left overnight. Acetic acid (0.4 ml of a 50% aqueous solution), 4.0 ml of Triton X-100 and 10 ml of Monofluor were added. These samples were left to stand in the dark overnight prior to determining the amount of radioactivity present by liquid scintillation counting.

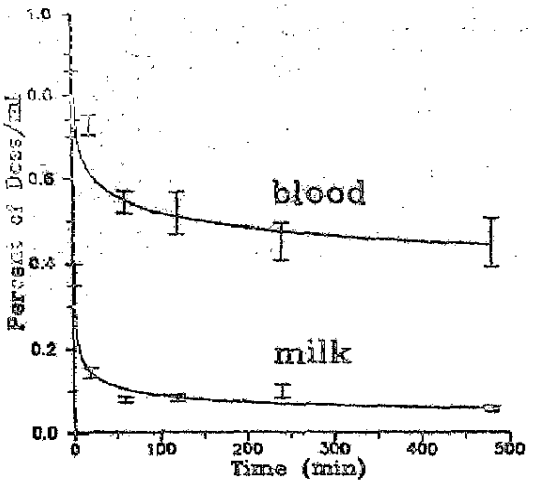

Fig. 1. Levels of radioactivity after i.v. administration of ^{14}C-labeled 4-ABP (60 mCi/mmol) in blood and milk of lactating dams 11 days post partum.

Table II. The influence of maternal transfer on the formation of DNA adducts in male and female pups[a]

Experimental no. of litter of 4-ABP treated dams[c]	No. of pups		fmol adducts/mg liver DNA[b]			
			Butanol extraction		Nuclease P_1	
	M	F	M	F	M	F
1	2	3	539	377	138	95
2	2	3	326	354	63	44
3	2	3	172	241	39	52
4	3	2	314	355	53	93
Average ± SE			338 ± 76	207 ± 31	74 ± 22	69 ± 13

[a]The control employed for this study consisted of four litters in which the dam was treated with corn oil as outlined in 'Materials and methods'. Of the five pups which were maintained in each control litter, the distribution of males and females was 2:3 for three with the fourth having three males and two females. Representative PEI–cellulose TLC maps of ^{32}P-labeled liver DNA from control pups is illustrated in Figures 2 and 3.
[b]Adduct levels were estimated by removing spots from the PEI-cellulose TLC maps and counting by liquid scintillation counting. The specific activity of the [γ-^{32}P]-used to label the adducts was determined by measuring the incorporation of ^{32}P into a known amount of deoxyadenosine 3′-monophosphate.
[c]Four male pups were also injected with 0.6 μmol of 4-ABP at 24 days of age. Control pups for this study consisted of pups treated with 20 μl dimethylsulfoxide. The liver DNA isolated from the treated pups had a similar profile of adducts on PEI-cellulose TLC plates as the pups obtained from the pooled liver samples obtained from the male and female pups from litters which were nursed from dams treated with 4-ABP. The livers of these pups contained 162 and 33 fmol of 4-ABP adducts/mg DNA as determined using the butanol extraction and nuclease P_1 procedures, respectively.

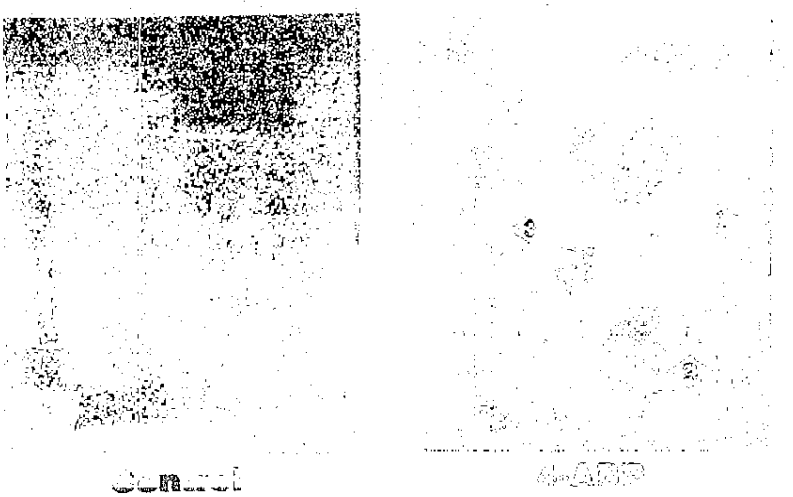

Fig. 2. Autoradiography of PEI-cellulose TLC maps from 11 μg of liver DNA of male pups from treated dams receiving corn oil (control) and 4-aminobiphenyl (4-ABP). Dams were treated on day 1 post-partum and then daily with corn oil or 4-ABP in corn oil for 2 weeks. ^{32}P-Postlabeling analysis was performed using the butanol extraction procedure with experimental procedures being performed as detailed in Materials and methods. Autoradiography was at −70°C for 12 h with intensifying screens. Arrows and numbers indicate locations referred to in Table IV. OR-origin.

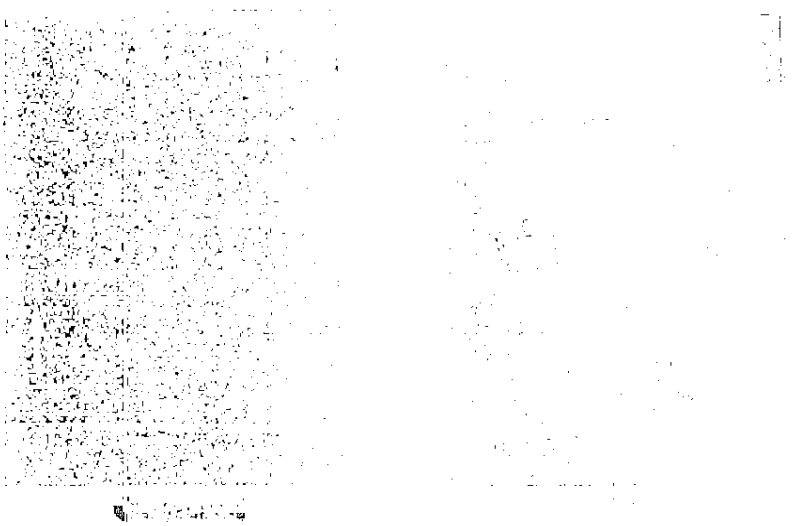

Fig. 3. Autoradiography of PEI-cellulose TLC maps from 11 μg of liver DNA of male pups from treated dams receiving corn oil (control) and 4-aminobiphenyl (4-ABP). Dams were treated on day 1 post-partum and then daily with corn oil or 4-ABP in corn oil for 2 weeks. ^{32}P-Postlabeling analysis was performed using the Nuclease P_1 procedure with experimental procedures being performed as detailed in Materials and methods. Autoradiography was at −70°C for 12 h with intensifying screens. OR-origin.

Bioassays on the formation of DNA adducts

Eight timed-pregnant Sprague–Dawley rats were divided into a experimental and a control group comprised of four dams each. Within 24 h after birth, each litter was reduced to five pups per litter. Dams in the experimental group were treated with 10 mg/kg 4-ABP in 250 μl corn oil. Dams in the control group received 250 μl corn oil/kg b.w. Both groups received their first s.c. injection within 24 h after giving birth, followed by daily injections until 14 days post partum. All animals were sacrificed when the pups were 15 days old. The livers of the pups were excised and pooled for each litter according to the sex. The number of male and female pups in each litter is provided in Table II. DNA was isolated from the pooled livers of the male and female pups as previously described (9). DNA adduct formation resulting from the maternal transfer of 4-ABP was determined as outlined below by ^{32}P-postlabeling.

The ability of male pups to form 4-ABP:DNA adducts after administration of a single i.p. injection of 4-ABP was also investigated. Four male pups at 24 days of age were injected with either 100 μg (0.5 μmol) of 4-ABP in dimethyl sulfoxide (20 μl) or dimethyl sulfoxide alone. These pups were sacrificed 24 h after treatment, livers excised and the DNA isolated as previously described (9). The extent of DNA adduct formation was determined by ^{32}P-postlabeling.

^{32}P-Postlabeling of liver DNA

DNA was digested to deoxyribonucleotides and postlabeled with ^{32}P as previously described using the Nuclease P_1 enrichment (13) and butanol extraction (16) procedures. Each sample was labeled using 24 μCi of [γ-^{32}P]-ATP. The specific activity of the radiolabeled ATP used ranged from 958–1900 Ci/mmol. No significant differences were observed in the levels of DNA adducts determined using ATP at either extremes within this range of specific activity. [γ-^{32}P]ATP with a specific activity of 958 Ci/mmol was used to obtain the results tabulated on the levels of the DNA adducts. Postlabeled 4-ABP:DNA adducts were chromatographed on PEI-cellulose TLC plates (Polygram cel PEI; Brinkmann Instruments Co., Westbury, NY) using a five directional developing system as described by Dunn and Stitch (17). The mobile phases employed for

Table III. Percent of radioactivity in milk associated with the ethyl acetate extract or protein precipitate[a]

Time (min)[b]	Ethyl acetate extract (%)	Protein precipitate (%)	Residual radioactivity[c]
< 1	43 ± 5	4 ± 1	53 ± 6
20	38 ± 3	6 ± 1	56 ± 4
60	30 ± 3	12 ± 1	61 ± 2
120	20 ± 3	16 ± 1	63 ± 1
240	19 ± 2	21 ± 1	60 ± 2
480	15 ± 1	16 ± 2	68 ± 2

[a] Values represent the mean ± SE.
[b] The number of milk samples used for these determinations were 3, 2, 4, 4, 3 and 4 at each time point of < 1, 20, 60, 120, 240 and 480 min, respectively.
[c] Residual radioactivity refers to the amount of radioactivity unaccounted for in either the ethyl acetate extract or protein precipitate mean ± SE and is presumed to be polar materials retained on the Chem Elut column.

Table IV. Relative distribution of liver DNA adducts of 4-ABP as determined after PEI-cellulose TLC

Location[a]	Route of exposure		
	Maternal transfer		Intraperitoneal injection
	(Percent of total DNA adducts detected)		
	Male	Female	Male
1	55	66	48
2	34	32	41
3	12	2	10

[a] DNA adducts indicated by arrows in Figure 2 were removed from the PEI-cellulose plate and the total amounts of radioactivity determined by liquid scintillation counting. The relative distribution of 4-ABP-DNA as indicated by arrows in Figure 3 were determined to be 65, 22 and 13% for location 1, 2 and 3, respectively.

each direction were as follows: D1: 2.3 M sodium phosphate, pH 3.8; D2: 3.75 M ammonium formate, pH 3.3; D3: 5.3 M lithium formate, pH 3.5, containing 8.5 M urea; D4: 1.1 M lithium chloride, 0.05 M Tris-Cl, pH 8.0, containing 8.5 M urea; D5: 1.7 M sodium phosphate, pH 6.0. The maps of DNA adduct were visualized by screen-enhanced autoradiography and the levels of adducts determined as previously described (13).

Results

The data on the transfer of 4-ABP and its metabolites from circulating blood to the milk of lactating rats at time intervals of < 1.0, 20, 60, 120, 240 and 480 min after dose administration are summarized in Table I. The difference in the extent to which 4-ABP and/or its metabolites persist in blood and milk is illustrated in Figure 1. Elimination of radioactivity from blood and milk was determined to be biphasic by nonlinear regression analysis, with data fitting the equation $C(t) = C_1 e^{-\alpha t} + C_2 e^{-\beta t}$. From this equation, half-lives were calculated for the rapid and slow phases of elimination. The half-lives of radioactivity elimination from blood and milk were calculated to be 24 and 8 min for the rapid phase and 38 and 21 h for the slow phase, respectively. The quantitative data outlined in Table I reflect not only the levels of 4-ABP and its metabolites in both milk and blood, but also the levels to which 4-ABP and its metabolites may be bound to protein or other macromolecules.

The extent to which 4-ABP and/or its metabolites could be extracted from milk absorbed on the Chem Elut tube at the various time points was also determined, see Table III. A greater than 95% recovery of unmetabolized 4-ABP from milk spiked with ^{14}C-labeled 4-ABP was obtained using Chem Elut tubes and ethyl acetate extraction. The percent of radioactivity in the ethyl acetate extract decreased with time from 43% to an average of 16% of the total amount present in the whole milk sample. The amount of radioactivity associated with the protein precipitate increased with time from an average of 4–21% of the total radioactivity detected in the milk after 4 h. The amount of radioactivity detected in the ethyl acetate extract and the protein precipitate, however, never exceeded 47% of the total detectable radioactivity in the whole milk sample. These data indicate that a substantial portion of the radioactivity in these milk samples was retained on the Chem Elut column.

The extent to which 4-ABP forms DNA adducts was examined in the livers of male and female pups exposed to 4-ABP and its metabolites through maternal transfer during nursing. The relative levels of adduct formation was determined using the butanol extraction and nuclease P_1 procedures (Table II). DNA adduct patterns were qualitatively similar for both male and female pups when analyzed by either the butanol extraction or nuclease P_1 postlabeling procedures. Representative TLC maps of the DNA adducts observed from pups nursed from a dam treated with 4-ABP are illustrated in Figures 2 and 3. The relative distribution of adducts in the specific zones indicated in Figure 2 are listed in Table IV. These data clearly indicate that the liver DNA of both male and female pups exposed to 4-ABP via maternal transfer are modified to similar extents. Similar profiles of ^{32}P-postlabeled DNA adducts were obtained from males injected with 4-ABP as compared to males and females exposed by maternal transfer to 4-ABP. The average level of adduct DNA present in the liver of pups 24 h after receiving a single i.p. injection of 100 μg of 4-ABP was less than that detected in pups nursed for two weeks from dams receiving 3–6 mg of 4-ABP daily by s.c. injection during this period (Table II).

Discussion

The results of the distribution of 4-ABP in lactating dams clearly indicate that 4-ABP and its metabolites can be transferred into milk. The elimination of 4-ABP and its metabolites from milk and blood was biphasic with the terminal elimination phase being more rapid from milk than blood, with half-lives of 21 and 38 h, respectively (Figure 1). Studies in laboratory animals and in humans have shown that 4-ABP, by the intermediacy of its *N*-nitroso metabolite, can bind to hemoglobin. The lifetime of red blood cells in rats and humans is approximately 30 and 120 days, respectively (18,19). The clearance of 4-ABP hemoglobin adducts in blood has also been shown to be similar to the lifetime of red blood cells in both rats and humans. Thus, the rate of elimination of ^{14}C-labeled 4-ABP from blood could be likely a result of the formation of such protein adducts. This factor may likely be responsible for the considerable difference observed in the biological half-lives of the terminal elimination phase of 4-ABP in blood as compared to milk. In addition, the concentration of 4-ABP and its metabolites was 2- to 3-fold higher in blood than in milk. This observation differs from that previously obtained with BaP, BNN and NNN. In the previous study performed on the distribution of these carcinogens into the milk of lactating Sprague–Dawley rats, it was shown that their concentration in blood after 1 h was similar to that in the milk. The observation that 4-ABP is detected in the blood of humans and in elevated levels in the blood of smokers suggests that 4-ABP could also be transferred into the milk of nursing mothers.

The percent of the dose of ^{14}C-labeled 4-ABP transferred into the milk after i.v. administration is lower than that observed previously for dams treated with BaP, NNK or NNN (9). In this previous study, higher levels of both NNK and NNN (150 μmol) as compared to 4-ABP were employed to evaluate their distribution into the milk. The effect of an increased dose of 4-ABP (16 μmol) indicate that a greater percentage of the administered dose can be detected in the milk and blood 1 h after dose administration.

Our data on 4-ABP distribution indicate that a large amount of the total material detected in milk is likely to be metabolites and/or polar derivatives of 4-ABP. That is, based upon recovered radioactivity, the amount of material accountable in the ethyl acetate extract and the protein precipitate never exceeded 47% of the total radioactivity detected in any of the whole milk samples. Therefore, the remaining 53% of the material is believed to be metabolites and/or polar derivatives of 4-ABP which are retained by the Chem Elut tubes. The presence of metabolites or polar derivatives is also suggested by control experiments in which greater than 95% of 4-ABP spiked into milk is recovered using identical analytical procedures. In addition, the amount of radioactivity associated with the ethyl acetate fraction decreased at longer times while material associated with the protein fraction and retained on the Chem Elut tubes increased. This shift in the ethyl acetate extractable material would be expected with increased overall metabolism of 4-ABP at the later time intervals.

The formation of 4-ABP:DNA adducts in newborn rat pups following the maternal transfer of 4-ABP was determined using the butanol extraction and nuclease P_1 enhancement variants of the ^{32}P-postlabeling procedure. Both procedures have been employed by others for the detection and quantitation of 4-ABP:DNA adducts (15). Since 4-ABP C8-substituted deoxyguanosine 3′-monophosphates are known to be dephosphorylated by nuclease P_1, the butanol extraction procedure is the preferred method. In this study, both analytical procedures were employed for comparative purposes. DNA adduct formation was detectable and qualitatively similar using both postlabeling procedures. Differences were observed in the levels of adducts detected as well as in the amount of background nucleotides present. The butanol extraction procedure resulted in two spots, in addition to the 4-ABP:DNA adducts, being detected in both experimental and control DNA digests. These spots accounted for ~0.29 pmol of material/mg DNA and were not detected when samples were treated with nuclease P_1 (Figure 3). ^{32}P Postlabeling of DNA digests hydrolyzed overnight, as opposed to 3 h, and subsequently analyzed using the butanol extraction procedure also produced these nuclease P_1 sensitive spots. These data suggest that these additional spots may be nucleotides, which are butanol extractable and not unhydrolyzed DNA. Quantitatively, the butanol extraction procedure resulted in a 3-fold greater adduct level than that determined using the nuclease P_1 procedure. The lower values obtained with the nuclease P_1 procedure may result from the dephosphorylation of C8-substituted deoxyguanosine 3′-monophosphate adducts. Although further studies are needed to determine the structural nature of the 4-ABP:DNA adducts detected in this study, it is evident that DNA adduct formation in newborn pups following maternal transfer is detectable by both the butanol extraction and the nuclease P_1 procedures.

Newborn mice have been used to bioassay the carcinogenic activity of 4-ABP (26). During each of the first 3 days of life, newborn mice were injected s.c. in the interscapular region with 200 μg of 4-ABP. After 52 weeks, 95% of the male and 17% of the female mice treated with 4-ABP developed hepatomas. Newborn male mice were clearly more susceptible to the carcinogenic effects of 4-ABP. The incidence of liver tumors observed among female mice as compared to controls in this bioassay was not statistically significant ($P < 0.05$). In our study, newborn rats were nursed from dams which were treated with 4-ABP in corn oil or with corn oil alone. DNA adduct formation in the liver was detectable in both male and female newborn rats. However, the levels of liver DNA adducts as determined by both postlabeling methods were not significantly different between male and female pups.

It is of interest that higher levels of DNA adducts were detected in rats exposed to 4-ABP by maternal transfer despite the estimation that they were exposed to a lower total dose than those receiving an i.p. injection of 100 μg of 4-ABP. One can estimate the total amount of 4-ABP or its metabolites which have been transferred to the newborns nursed from treated dams in this study. Since each dam received 4.0 mg of 4-ABP daily for 2 weeks (56 mg, total dose) and assuming that 0.2% of the dose (based on milk transfer data) was transferred to the 5 pups maintained in each litter, one could calculate that each pup would receive a total dose of ~22 μg of 4-ABP. Therefore, pups receiving an i.p. injection of 4-ABP were exposed to 4-fold higher levels of 4-ABP than nursing pups. It should be noted that several factors such as route of administration, dose frequency, and age, however, preclude a direct comparison of the levels of liver DNA adducts formed between rats exposed to 4-ABP by maternal transfer and rats which received a single i.p. injection.

The maternal transfer of several carcinogens during nursing has been shown to result in the development of tumors in the offspring (21–23). The extent of DNA modification which occurred to the nursing pups by exposing the dams to 4-ABP in this study indicates that 4-ABP or its genotoxic metabolites are being transferred to pups. The detection of DNA adducts in 24 day old pups injected with 4-ABP indicates that these young rats can effectively activate 4-ABP to a genotoxic agent. Thus, the adducts detected in the pups nursed from dams exposed to 4-ABP could result from exposure to either 4-ABP or a genotoxic metabolite of 4-ABP. The potential susceptibility of infants to carcinogen exposure raises concern that exposure of nursing mothers to several of the known carcinogens in tobacco or tobacco smoke may result in the partial transfer of these genotoxic agents to nursing infants.

Acknowledgements

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